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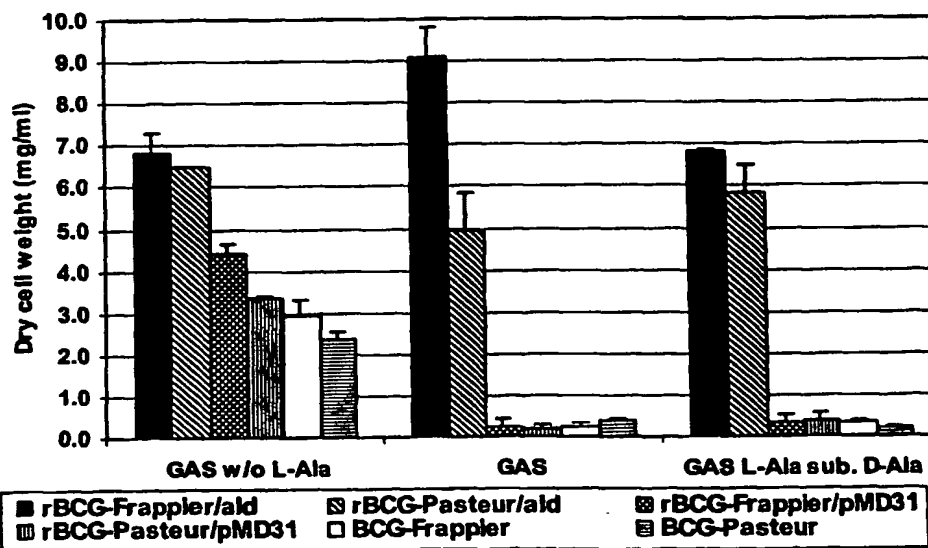
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(54) Title: TUBERCULOSIS VACCINES INCLUDING RECOMBINANT BCG STRAINS EXPRESSING ALANINE DEHY-  
DROGENASE, SERINE DEHYDRATASE AND/OR GLUTAMINE SYNTHETASE



(57) Abstract: The invention relates to a live recombinant *Mycobacterium bovis*-BCG strain comprising a nucleic acid capable of expression, the nucleic acid encoding at least one protein or polypeptide that exhibits alanine dehydrogenase activity, glutamine synthetase activity, or serine dehydratase activity.



— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

*For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*

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# INTERNATIONAL SEARCH REPORT

International Application No  
PCT/ 03/00566

|  |  |                                  |
|--|--|----------------------------------|
| <b>A. CLASSIFICATION OF SUBJECT MATTER</b><br>IPC 7 C12N15/53 C12N15/60 C12N1/21 G01N33/53 A61K38/43<br>A61K39/04  |  |                                  |
| According to International Patent Classification (IPC) or to both national classification and IPC  |  |                                  |
| <b>B. FIELDS SEARCHED</b><br>Minimum documentation searched (classification system followed by classification symbols)<br>IPC 7 C12N G01N A61K   |  |                                  |
| Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched  |  |                                  |
| Electronic data base consulted during the international search (name of data base and, where practical, search terms used)<br>EPO-Internal, WPI Data, PAJ, Sequence Search, BIOSIS, EMBASE   |  |                                  |
| <b>C. DOCUMENTS CONSIDERED TO BE RELEVANT</b>  |  |                                  |
| Category *   | Citation of document, with indication, where appropriate, of the relevant passages   | Relevant to claim No.            |
| X<br><br>A   | WO 98/32862 A (FLOHE, L.; SINGH, M.; HUTTER, B. & KOLK, A.)<br>30 July 1998 (1998-07-30)<br>the whole document<br>see especially:<br>page 40, line 17 - page 44, line 17<br>page 45, line 17 - line 28<br>and<br>pages 51-55; claims 1-5,8-11<br><div style="text-align: center;">-----<br/>-/--</div> | 1,3,4,<br>6-16,20<br><br>2,17-19 |
| <div style="display: flex; justify-content: space-between;"> <span><input checked="" type="checkbox"/> Further documents are listed in the continuation of box C.</span> <span><input checked="" type="checkbox"/> Patent family members are listed in annex.</span> </div>  |  |                                  |
| * Special categories of cited documents :<br><div style="display: flex;"> <div style="flex: 1;"> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> </div> <div style="flex: 1;"> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.</p> <p>"&amp;" document member of the same patent family</p> </div> </div> |  |                                  |
| Date of the actual completion of the international search  | Date of mailing of the international search report   |                                  |
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# INTERNATIONAL SEARCH REPORT

International Application No  
PCT/ 03/00566

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages  | Relevant to claim No. |
|------------|---|-----------------------|
| A          | <p>WO 01/078774 A (REGENTS OF THE UNIVERSITY OF CALIFORNIA)<br/>25 October 2001 (2001-10-25)<br/>abstract<br/>page 10, line 5 - page 11, line 33<br/>58 kDa major extracellular protein of Mycobacterium sp.<br/>page 14, line 15 - page 15, line 19<br/>page 29 - page 33; claims 1-29</p>   | 1-4,6-20              |
| L          | <p style="text-align: center;">-----</p> <p>HARTH, G. ET AL.: "Glutamine synthetase of Mycobacterium tuberculosis: Extracellular release and characterization of its enzymatic activity"<br/>PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA,<br/>vol. 91, no. 20,<br/>27 September 1994 (1994-09-27), pages 9342-9346, XP002264578<br/>cited to provide background information on relevant subject-matter disclosed in WO0178774<br/>the whole document</p> | 1-4,6-20              |
| A          | <p style="text-align: center;">-----</p> <p>ANDERSEN, P.: "TB vaccines: progress and problems"<br/>TRENDS IN IMMUNOLOGY,<br/>vol. 22, no. 3, March 2001 (2001-03),<br/>pages 160-168, XP004255881<br/>cited in the application<br/>the whole document<br/>see especially:<br/>page 165, column 1, line 21 - column 2, line 17</p>   | 1-4,6-20              |
| A          | <p style="text-align: center;">-----</p> <p>WO 00/11214 A (BOARD OF TRUSTEES OF THE LELAND STANFORD JUNIOR UNIVERSITY)<br/>2 March 2000 (2000-03-02)<br/>the whole document<br/>see especially:<br/>page 3, line 29 - line 32<br/>page 23, line 1 - page 25, line 24<br/>and<br/>page 31; claims 8-10</p>   | 1-4,6-20              |
| A          | <p style="text-align: center;">-----</p> <p>IORI, F. ET AL.: "Long-term maintenance bacille calmette-guérin therapy in high-grade superficial bladder cancer"<br/>UROLOGY,<br/>vol. 59, no. 3, March 2002 (2002-03),<br/>pages 414-418, XP002264579<br/>the whole document</p> <p style="text-align: center;">-----</p> <p style="text-align: center;">-/--</p>   | 1-4,6,7,<br>17-19     |

# INTERNATIONAL SEARCH REPORT

International Application No

PCT/CA 03/00566

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages  | Relevant to claim No. |
|------------|---|-----------------------|
| A          | HARTH, G. & HORWITZ, M.A.: "An Inhibitor of Exported Mycobacterium tuberculosis Glutamine Synthetase Selectively Blocks the Growth of Pathogenic Mycobacteria in Axenic Culture and in Human Monocytes: Extracellular Proteins as Potential Novel Drug Targets"<br>JOURNAL OF EXPERIMENTAL MEDICINE, vol. 189, no. 9, 3 May 1999 (1999-05-03), pages 1425-1435, XP002264580<br>page 1429, column 1, line 28 - column 2, line 15<br>page 1431; figure 3E | 1-4,6-20              |
| P,X        | -----<br>CHEN, J.M. ET AL.: "Mycobacterium bovis BCG Vaccines Exhibit Defects in Alanine and Serine Catabolism"<br>INFECTION AND IMMUNITY, vol. 71, no. 2, February 2003 (2003-02), pages 708-716, XP002264581<br>the whole document<br>-----   | 1-4,6-20              |

# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/CA 03/00566

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:  
**Although claims 13-19 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.**
2. ☒ Claims Nos.: **5 (completely) and 6-20 (all partially)**  
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:  
**see FURTHER INFORMATION sheet PCT/ISA/210**
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

**see additional sheet**

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:  
**1-20 (all partially)**

### Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.  
☐ No protest accompanied the payment of additional search fees.

## FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-20 (all partially)

A live recombinant Mycobacterium bovis-BCG strain comprising a nucleic acid capable of expression, the nucleic acid: encoding at least one protein or polypeptide that exhibits alanine dehydrogenase activity, encoding at least one protein or polypeptide selected from the group consisting of alanine dehydrogenase [SEQ ID NO: 1, SEQ ID NO: 2], comprises all or part of at least one nucleic acid molecule selected from the group consisting of [SEQ ID NO: 1], or comprises a sequence having at least 60% sequence identity to at least one nucleic acid molecule selected from the group consisting of [SEQ ID NO: 1], a pharmaceutical composition comprising said live recombinant Mycobacterium bovis-BCG strain, a vaccine or immunogenic composition for treatment or prophylaxis of a mammal against challenge by mycobacteria or cancer comprising said live recombinant Mycobacterium bovis-BCG strain, a test kit comprising said live recombinant Mycobacterium bovis-BCG strain;

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2. claims: 1-20 (all partially)

idem as subject 1, but limited to glutamine synthetase and sequences [SEQ ID NOS: 7, 9, 11, 13 and SEQ ID NOS: 8, 10, 12, 14];

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3. claims: 1-20 (all partially)

idem as subject 1, but limited to L-serine dehydratase and sequences [SEQ ID NO: 5, SEQ ID NO: 6];

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4. claims: 21-27 (all completely)

a media composition for inhibiting the growth of Mycobacterium bovis-BCG comprising alanine or serine as the only nitrogen source for growth, a method for inhibiting the growth of Mycobacterium bovis-BCG comprising culturing a sample comprising Mycobacterium in a selective media wherein the selective media comprises alanine or serine as the only nitrogen source for growth;

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5. claims: 28, 29 (both completely)

a method of culturing Mycobacterium bovis-BCG comprising culturing a sample comprising Mycobacterium in differential media wherein the differential media comprises histidine.

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FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

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Continuation of Box I.2

Claims Nos.: 5 (completely) and 6-20 (all partially)

Present claim 5 and dependent claims 6-20 relate to a nucleic acid molecule defined by reference to a desirable characteristic or property, namely "... has undergone modification".

The claims cover all nucleic acid molecules having this characteristic or property, whereas the application provides no support within the meaning of Article 6 PCT and/or no disclosure within the meaning of Article 5 PCT for such a nucleic acid molecule. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search is impossible. Independent of the above reasoning, the claims also lack clarity (Article 6 PCT). An attempt is made to define the nucleic acid molecule by reference to a result to be achieved. Again, this lack of clarity in the present case is such as to render a meaningful impossible. Consequently, no search has been carried out for the subject-matter of claim 5 and for subject-matter of claims 6-20 as far as referring to claim 5.

The applicant's attention is drawn to the fact that claims relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure. If the application proceeds into the regional phase before the EPO, the applicant is reminded that a search may be carried out during examination before the EPO (see EPO Guideline C-VI, 8.5), should the problems which led to the Article 17(2) declaration be overcome.



# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/JP 03/00566

| Patent document<br>cited in search report |   | Publication<br>date | Patent family<br>member(s)   | Publication<br>date  |
|---|---|---------------------|--|--|
| WO 9832862                                | A | 30-07-1998          | AU 6097998 A<br>WO 9832862 A2  | 18-08-1998<br>30-07-1998   |
| WO 0178774                                | A | 25-10-2001          | US 6471967 B1<br>AU 5355801 A<br>CA 2406225 A1<br>CN 1437479 T<br>EP 1274453 A2<br>WO 0178774 A2<br>US 2003124135 A1<br>US 2004009184 A1 | 29-10-2002<br>30-10-2001<br>25-10-2001<br>20-08-2003<br>15-01-2003<br>25-10-2001<br>03-07-2003<br>15-01-2004 |
| WO 0011214                                | A | 02-03-2000          | US 6291190 B1<br>AU 5394699 A<br>EP 1108060 A1<br>WO 0011214 A1<br>US 2004018574 A1<br>US 2002176873 A1                                  | 18-09-2001<br>14-03-2000<br>20-06-2001<br>02-03-2000<br>29-01-2004<br>28-11-2002                             |